

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>A61K 9/14, 9/51, 49/04</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/22766</b> <b>(43) International Publication Date:</b> 1 August 1996 (01.08.96)
<b>(21) International Application Number:</b> PCT/US95/15173 <b>(22) International Filing Date:</b> 8 December 1995 (08.12.95)  <b>(30) Priority Data:</b> 08/378,022 24 January 1995 (24.01.95) US  <b>(71) Applicant (for all designated States except US):</b> NANOSYSTEMS L.L.C. [US/US]; 1250 South Collegeville Road, Building 1, Collegeville, PA 19426 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> WONG, Sui-Ming [US/US]; 159 Derr Drive, Collegeville, PA 19426 (US). NEWINGTON, Ian, M. [GB/GB]; 11 Ashtree Walk, Hazlemere, High Wycombe, Buckinghamshire HP15 7TG (GB). LIVERSIDGE, Elaine, M. [US/US]; 258 Colwyn Terrace, West Center, PA 19680 (US). McINTIRE, Gregory, L. [US/US]; 113 Piedmont Road, West Chester, PA 19382 (US). PITT, Alan, R. [GB/GB]; 49 St. Albans Road, Sandridge, St. Albans, Herts AL4 9LE (GB). SHAW, Jack, Michael [US/US]; 2738 Carsins Run Road, Aberdeen, MD 21001 (US).  <b>(74) Agent:</b> WEST, Paul, B.; Ladas & Parry, 26 West 61st Street, New York, N.Y. 10023 (US).		<b>(81) Designated States:</b> AL, AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SULFATED NONIONIC BLOCK COPOLYMER SURFACTANTS AS STABILIZER COATINGS FOR NANOPARTICLE COMPOSITIONS  <b>(57) Abstract</b>  This invention provides a composition comprised of nanoparticles containing a therapeutic or diagnostic agent having a block copolymer linked to at least one anionic group as a surface modifier adsorbed on the surface thereof and a method of making such nanoparticles. The compositions exhibit unexpectedly improved autoclave stability, reduced macrophage uptake, improved toxicological profiles and facilitate particle size reduction such that milling time can be reduced and/or sterile filtration of the nanoparticles can be accomplished.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

-1-

SULFATED NONIONIC BLOCK COPOLYMER SURFACTANTS AS  
STABILIZER COATINGS FOR NANOPARTICLE COMPOSITIONS

FIELD OF THE INVENTION

5 This invention relates to therapeutic and  
diagnostic compositions containing a surfactant and to  
a method for the preparation thereof.

BACKGROUND OF THE INVENTION

10 U.S. Patent No. 5,145,684 discloses particles  
of a drug substance having a surface modifier absorbed  
on the surface thereof and methods for the preparation  
thereof by wet grinding. These particles have  
demonstrated significant pharmaceutical utility.  
Suitable surface modifiers described include various  
polymers. The surface modifiers disclosed include  
15 Pluronic F68 and F108, which are block copolymers of  
ethylene oxide and propylene oxide; Tetronic 908, which  
is a tetrafunctional block copolymer derived from  
sequential addition of propylene oxide and ethylene  
oxide to ethylene diamine; sodium dodecylsulfate,  
20 dialkyl esters of sodium sulfosuccinic acid such as  
Aerosol OT™, sodium lauryl sulfate, and Triton™ X-200.

25 U.S. Patent No. 5,318,767 discloses X-ray  
contrast compositions comprising particles of an X-ray  
contrast agent having a surface modifier absorbed on  
the surface thereof and methods for the preparation  
thereof by wet grinding. The above-noted surface  
modifiers are also disclosed as being useful therein.  
These X-ray contrast compositions have demonstrated  
remarkable utility in X-ray medical diagnostic imaging  
30 procedures.

U.S. Patent No. 5,340,564 discloses  
therapeutic and diagnostic compositions with Olin-10 G,

-2-

i.e., p-isononylphenoxypoly(glycidol) having improved autoclave stability.

However, sterilization of therapeutic and diagnostic agents in nanoparticulate form stabilized by a surface modifier is difficult. Filtration using a filter of 0.22  $\mu\text{m}$  mesh size is sufficient to remove most bacteria, but the nanoparticles, due to their sizes, cannot be sterile filtered without accounting for substantial drug losses. Conventional autoclaving (steam heat) at 121°C generally results in substantial increase in particle size, rendering the resulting particles unusable. One possible explanation is that the aggregation of nanoparticles upon heating is related to the phase separation of the surface modifier (surfactant) at or below the sterilization temperature where the bound surfactant molecules are likely to dissociate from the nanoparticles and precipitate, leaving the nanoparticles unprotected. The unprotected nanoparticles can then aggregate into clusters of particles. Upon cooling, the surfactant redissolves into the solution, which then coats the aggregated particles and prevents them from dissociating into smaller ones.

Additional difficulties of some of the above-described surfactants relate to biological usage and wet grinding method.

For example, some of the above-described surfactants have demonstrated less than superior toxicological profiles such as T908, DOSS, Pluronic S127 and Olin 10G. In addition, these prior art surfactant coatings, when adsorbed to nanoparticles, on some occasions, have exhibited less than desirable uptake of nanoparticles by macrophages.

-3-

Moreover, the wet grinding methods described in the patents noted above often entail grinding for days or even weeks which can be undesirable, e.g., from the standpoint of process scale-up.

5                   Consequently, it would be highly desirable to provide surfactant coatings for nanoparticles which provide improved resistance to particle size increase during autoclaving, exhibit improved toxicological profiles, minimize macrophage uptake and facilitate  
10 particle size reduction such that milling time can be reduced and/or sterile filtration of the nanoparticles can be accomplished without substantial particle losses.

#### SUMMARY OF THE INVENTION

15                   We have discovered surfactant coatings for nanoparticles which provide unexpected resistance to nanoparticle size growth during autoclaving, inhibit macrophage uptake, has an improved safety profile, and facilitate particle size reduction.

20                   More specifically, in accordance with this invention, there is provided a composition comprised of nanoparticles containing a therapeutic or diagnostic agent having a block copolymer linked to at least one anionic group as a surface modifier absorbed on the  
25 surface thereof.

                  It is an advantageous feature of this invention that surfactants are provided for nanoparticle compositions which allow autoclaving of the nanoparticles without the concomitant growth in  
30 particle size and which exhibit an improved toxicological profile.

-4-

It is another advantageous feature of this invention that nanoparticle compositions are provided which minimize macrophage uptake.

Yet another advantageous feature of this invention is that nanoparticle compositions are provided in a narrow particle size distribution.

Still another advantageous feature of this invention is that a surfactant coating is provided for nanoparticles which facilitates particle size reduction, thus reducing milling time and potentially enabling sterile filtration of the nanoparticles to be accomplished without substantial particle losses.

These and other advantages will become readily apparent upon reference to the following description of preferred embodiments.

#### DESCRIPTION OF PREFERRED EMBODIMENTS

This invention is described hereinafter primarily in connection with nanoparticles containing a therapeutic or diagnostic agent having a block copolymer linked to at least one anionic group as a surface modifier adsorbed on the surface thereof.

Surface modifiers useful herein are block copolymers linked to at least one anionic group. The polymers contain at least one, and preferably two, three, four or more anionic groups per molecule. Preferred anionic groups include sulfate, sulfonate, phosphonate, phosphate and carboxylate groups. The anionic groups are covalently attached to the nonionic block copolymer. The nonionic sulfated polymeric surfactant has a molecular weight of 1,000-50,000, preferably 2,000-40,000 and more preferably 3,000-30,000. In preferred embodiments, the polymer comprises at least about 50%, and more preferably, at

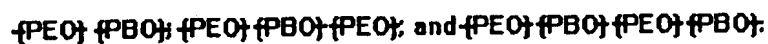
-5-

least about 60% by weight of hydrophilic units, e.g., alkylene oxide units. The reason for this is that the presence of a major weight proportion of hydrophilic units confers aqueous solubility to the polymer. The  
5 block copolymers useful herein are known compounds and/or can be readily prepared by techniques well known in the art.

A preferred class of block copolymers useful in preparing surface modifiers for use herein includes  
10 sulfated block copolymers of ethylene oxide and propylene oxide. These block copolymers, in an unsulfated form, are generically called poloxamers and are commercially available as Pluronic<sup>TM</sup>. Specific examples of the unsulfated block copolymers include  
15 F68, F108, and F127.

Another preferred class of block copolymers useful herein include tetrafunctional block copolymers derived from sequential addition of propylene oxide and ethylene oxide to ethylene diamine. These polymers, in  
20 an unsulfated form, are commercially available as Tetronics<sup>TM</sup>.

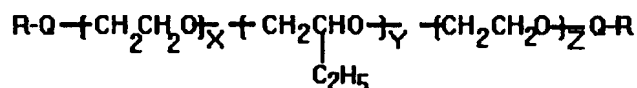
Another preferred class of block copolymers contain at least one polyethylene oxide (PEO) block as the hydrophilic portion of the molecule and at least  
25 one polybutylene oxide (PBO) block as the hydrophobic portion. Particularly preferred copolymers of this class are diblock, triblock, and higher block copolymers of ethylene oxide and butylene oxide, such as are represented, for example, by the following  
30 structural formula:



Highly preferred surface modifiers include triblock copolymers of the structure  $\text{-(PEO)-(PBO)-(PEO)-}$  having

-6-

molecular weights of 3800 and 5000 which are commercially available from Dow Chemical, Midland, Michigan, and are hereinafter referred to as B20-3800 and B20-5000. These surface modifiers contain about  
 5 80% by weight PEO. In a preferred embodiment, the surface modifier is a triblock polymer linked to an anionic group having the structure:



wherein Q is an anionic group, R is H or a metal cation  
 10 such as Na<sup>+</sup>, K<sup>+</sup> and the like, X is 15-700, Y is 5-200 and Z is 15-700.

While the applicants do not wish to be bound by theoretical mechanisms, it is believed that the presence of the anionic group increases the ionic  
 15 character and polarity of the surface modifier and provides both electrostatic and steric stabilization, thus reducing the tendency of the surface modifier to phase separate during steam heat autoclaving.

The surface modifier useful herein can be prepared by techniques known in the art. Thus, the  
 20 anionic group can be linked to the block copolymer using conventional linking technology. For example, the sulfated nonionic block copolymers useful herein can be prepared from the unsulfated form of the  
 25 polymers by conventional sulfation techniques. For example, sulfated F68, F108, F127 ... can be prepared by dissolving the unsulfated nonionic block copolymer in a suitable solvent, e.g., 1,2-dichloroethane and contacting the dissolved polymer with a source of  
 30 sulfate ions, e.g., chlorosulphonic acid. Suitable solvents include tetrahydrofuran, dichloromethane, chloroform, dioxane and 1,2-dimethoxyethane. Suitable



-7-

sources of sulfate ions include sulfur trioxide and sulfur trioxide-pyridine complex. Sulfation can take place at temperatures from about 25°C to 50°C and higher. Contact times can range from seconds to several hours. The reaction can take place at atmospheric pressure. However, higher and lower reaction pressures are contemplated. In a similar manner, block copolymers can be reacted with a source of sulfonate, phosphate or phosphonate ions to form the surface modifiers useful herein. In the case of carboxylate anions, direct oxidation or the addition of a carboxyl-containing compound such as a bromoacetate is employed.

The nanoparticles useful in the practice of this invention can be prepared according to the methods disclosed in U.S. Patent No. 5,145,684 and U.S. Patent No. 5,318,767.

Briefly, a method for the preparation of a nanoparticle composition according to this invention includes the steps of introducing a diagnostic or therapeutic agent, a liquid medium, grinding media, and optionally, a surface modifier into a grinding vessel; wet grinding to reduce the particle size of the agent to less than about 1000 nm; and separating the particles and the liquid medium from the grinding vessel and grinding media, for example, by suction, filtration or evaporation. If the surface modifier is not present during wet grinding, it can be admixed with the particles thereafter. The liquid medium, most often water, can serve as the pharmaceutically acceptable carrier. The method preferably is carried out under aseptic conditions.

A general procedure for preparing the particles useful in the practice of this invention follows. The therapeutic or diagnostic agent selected

-8-

is obtained commercially and/or prepared by techniques known in the art, in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse therapeutic or diagnostic substance selected  
5 be less than about 100  $\mu\text{m}$  as determined by sieve analysis. If the coarse particle size of that agent is greater than about 100  $\mu\text{m}$ , then it is preferred that the coarse particles of the therapeutic or diagnostic agent be reduced in size to less than 100  $\mu\text{m}$  using a  
10 conventional milling method such as airjet or fragmentation milling.

The coarse therapeutic or diagnostic agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The  
15 concentration of the therapeutic or diagnostic agent in the liquid medium can vary from about 0.1-60%, and preferably is from 5-30% (2/2). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier  
20 can vary from about 0.1 to 90%, and preferably is 1-75%, more preferably 2-50% and most preferably 5-45% by weight based on the total combined weight of the drug substance and surface modifier. The apparent viscosity of the premix suspension is preferably less than about  
25 1000 centipoise.

The premix can be used directly by wet grinding to reduce the average particle size in the dispersion to less than about 1000 nm. It is preferred that the premix be used directly when a ball mill is  
30 used for attrition. Alternatively, the therapeutic or diagnostic agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogenous dispersion is observed in which  
35 there are no large agglomerates visible to the naked

-9-

eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

Wet grinding can take place in any suitable dispersion mill, including, for example, a ball mill, an attritor mill, a vibratory mill, a planetary mill and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles of the invention with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. However, media with higher density, e.g., glass (2.6 g/cm<sup>3</sup>), zirconium silicate (3.7 g/cm<sup>3</sup>), and zirconium oxide (5.4 g/cm<sup>3</sup>), are generally preferred for more efficient milling. Zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of therapeutic or diagnostic compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium, are believed to be

-10-

useful. In addition, polymeric media having a density typically from 1 to 2 g/cm<sup>3</sup> are also expected to be useful under certain milling conditions. The grinding media can be a polymeric media such as described in  
5 European Patent Application No. 600,528.

The attrition time can vary widely and depends primarily upon the particular wet grinding mill selected. For ball mills, processing times of up to five days or longer may be required. On the other  
10 hand, processing times of less than 1 day (residence times of about one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a  
15 temperature which does not significantly degrade the therapeutic or diagnostic agent. Processing temperature of less than about 30-40°C are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method  
20 is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Processing  
25 pressures up to about 20 psi (1.4 kg/cm<sup>2</sup>) are typical of media milling.

The surface modifier, if not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix. Thereafter,  
30 the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a

-11-

frequency of 20-80 kHz for a time of about 1 to 120 seconds.

The relative amount of therapeutic or diagnostic agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular therapeutic or diagnostic agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the hydrophilic lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc. The surface modifier preferably is present in an amount of about 0.1-10 mg per square meter surface area of the therapeutic or diagnostic agent. The surface modifier can be present in an amount of 0.1-90%, preferably 1-75%, more preferably 2-50%, and most preferably 5-45% by weight based on the total weight of the dry particle. The surface modifier preferably is present in an amount exceeding the critical miscelle concentration.

Therapeutic and diagnostic agents useful in the composition of the present invention include those disclosed in U.S. Patent No. 5,145,684, and U.S. Patent No. 5,318,767 whose disclosures are incorporated herein by reference. Preferred diagnostic agents include the X-ray imaging agent ethyl 3,5-diacetamido-2,4,6-triiodobenzoate; 6-ethoxy-6-oxohexyl-3,5-bis(acetamido)-2,4,6-triiodobenzoate; ethyl-2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)butyrate; ethyl diatrizoxyacetate; ethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)propionate; N-ethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy) acetamide; isopropyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy) acetamide; diethyl 2-(3,5-

-12-

bis(acetamido)-2,4,6-triiodobenzoyloxy) malonate; and ethyl 2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy) phenylacetate. Suitable diagnostic agents are also disclosed in U.S. Patent No. 5,260,478; U.S. Patent No. 5,264,610; U.S. Patent No. 5,322,679 and U.S. Patent No. 5,300,739.

A method for preparation of a nanoparticle composition according to this invention includes the steps of introducing a therapeutic or diagnostic agent, a liquid medium, grinding media, and optionally, a surface modifier into a grinding vessel; wet grinding to reduce the particle size of the therapeutic or diagnostic agent to less than about 1000 nm; and separating the particles and the liquid medium from the grinding vessel and grinding media, for example, by suction, filtration or evaporation. If the surface modifier is not present during wet grinding, it can be admixed with the particles thereafter. The liquid medium, most often water, can serve as the pharmaceutically acceptable carrier. The method can be carried out under aseptic conditions. Thereafter, the nanoparticle composition preferably is subjected to a sterilization process.

As noted elsewhere herein, sterile filtration will not provide adequate sterilization for nanoparticles without causing significant loss of active material. Although the compositions of this invention can be sterile filtered, other methods of sterilization can also be employed. For example, steam or moist heat sterilization at temperatures of about 121°C for a time period of about 20 minutes can be used. At altitudes near sea level, such conditions are attained by using steam at a pressure of 15 pounds per square inch (psi) in excess of atmospheric pressure.

-13-

Dry heat sterilization may also be performed, although the temperatures used for dry heat sterilization are typically 160°C for time periods of 1 to 2 hours.

5                   It is an advantageous feature of this invention that nanoparticle compositions are provided having unexpectedly improved autoclave stability in the substantial absence of secondary stability enhancing agents. Nonetheless, the therapeutic or diagnostic  
10 agent in the form of surface modified nanoparticles can be associated with a cloud point modifier to further enhance stability during steam heat autoclaving, i.e., the cloud point modifier can further reduce particle aggregation during heat sterilization. Preferred cloud  
15 point modifiers include nonionic cloud point modifiers, such as those described in U.S. Patent No. 5,346,702 including polyethylene glycols such as PEG 400, propylene glycol, ethanol, hydroxypropylcyclodextrin and glycerol; ionic cloud point modifiers, such as  
20 those described in U.S. Patent No. 5,298,262 including dialkylesters of sodium sulfosuccinic acid such as the dioctylester of sodium sulfosuccinic acid (DOSS); and charged phospholipids, such as those described in U.S. Patent No. 5,336,507 including diacylphosphatidyl  
25 glycerol and dimyristoylphosphatidyl glycerol. The cloud point modifier can be present in an amount of 0.005-50%, preferably 0.01-30% and more preferably 0.05-20% by weight based on the total weight of the nanoparticle composition.

30                   Therapeutic and diagnostic compositions according to this invention include the particles described above and a pharmaceutically acceptable carrier therefor. Suitable pharmaceutically acceptable carriers are well known to those skilled in the art.  
35 These include non-toxic physiologically acceptable

-14-

carriers, adjuvants or vehicles for parenteral injection, for oral administration in solid or liquid form, for rectal administration, and the like. A method of treating a mammal in accordance with this invention comprises the step administering to the mammal in need of treatment an effective amount of the above-described therapeutic composition. The selected dosage level of the therapeutic substance for treatment is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore, depends upon the particular drug substance, the desired therapeutic effect, on the route of administration, on the desired duration of treatment and other factors.

15 In a preferred embodiment, the diagnostic compound is an X-ray contrast agent, such as an iodinated X-ray contrast agent. Thus, the diagnostic compositions of this invention include an X-ray contrast composition comprising particles containing an X-ray contrast agent and a physiologically acceptable carrier therefor. For example, the particles can be dispersed in an aqueous liquid which serves as the carrier for the X-ray contrast agent. Other suitable carriers include liquid carriers such as mixed aqueous and nonaqueous solvents, such as alcohol; gels; gases; such as air; and powders.

The X-ray contrast compositions can comprise from about 1-99.9, preferably 2-45 and more preferably 10-30% by weight of the above-described particles, the remainder of the composition being the carrier, additives and the like. Compositions up to about 100% by weight of the particles are contemplated when the composition is in a lyophilized form.

The X-ray contrast composition can contain one or more conventional additives used to control



-15-

and/or enhance the properties of the X-ray contrast agent. For example, thickening agents such as dextran or human serum albumin, buffers, viscosity regulating agents, suspending agents, peptizing agents, anti-clotting agents, mixing agents, and other drugs and the like can be added. A partial listing of certain specific additives includes gums, sugars such as dextran, human serum albumin, gelatin, sodium alginate, agar, dextrin, pectin and sodium carboxymethyl cellulose. Such additives, surface active agents, preservatives and the like can be incorporated into the compositions of the invention.

A method for diagnostic imaging for use in medical procedures in accordance with this invention comprises administering to the body of a test subject the above-described X-ray contrast composition. In addition to human patients, the test subject can include mammalian species such as rabbits, dogs, cats, monkeys, sheep, pigs, horses, bovine animals and the like. Thereafter, at least a portion of the body containing the administered contrast agent is exposed to X-rays to produce an X-ray image pattern. The image pattern can then be visualized. For example, any X-ray visualization technique, preferably, a high contrast technique such as computed tomography, can be applied in a convention manner. Alternatively, the image pattern can be observed directly on an X-ray sensitive phosphor screen-silver halide photographic film combination.

The compositions of this invention can be administered by a variety of routes depending on the type of procedure and the anatomical orientation of this tissue being examined. Suitable administration routes include intravascular (arterial or venous) administration by catheter, intravenous injection,

-16-

rectal administration, subcutaneous administration,  
intramuscular administration, intralesional  
administration, intrathecal administration,  
intracisternal administration, oral administration,  
5 administration via inhalation, administration directly  
into a body cavity, e.g., arthrography, and the like.  
In addition to preferred applications, i.e., for blood  
pool, liver, spleen and lymph node imaging, the X-ray  
contrast compositions of this invention are also  
10 expected to be useful as an angiographic contrast  
media, urographic contrast media, myelographic contrast  
media, gastrointestinal contrast media,  
cholecystographic and cholangiographic contrast media,  
arthrographic contrast media, hysterosalpingographic  
15 contrast media, oral contrast media and bronchographic  
contrast media.

The dose of the contrast agent to be  
administered can be selected according to techniques  
known to those skilled in the art such that a  
20 sufficient contrast enhancing effect is obtained.  
Typical doses can range from 50 to 350 mg of iodine per  
kilogram of body weight of the subject for many imaging  
applications. For some applications, e.g.,  
lymphography, lower doses, e.g., 0.5-20 mg I/kg, can be  
25 effective.

The X-ray contrast composition can contain  
one or more conventional additives used to control  
and/or enhance the properties of the X-ray contrast  
agent. For example, thickening agents such as dextran  
30 or human serum albumin, buffers, viscosity regulating  
agents, suspending agents, peptizing agents, anti-  
clotting agents, mixing agents, and other drugs and the  
like can be added. A partial listing of certain  
specific additives includes gums, sugars such as  
35 dextran, human serum albumin, gelatin, sodium alginate,

-17-

agar, dextrin, pectin and sodium carboxymethyl cellulose. Such additives, surface active agents, preservatives and the like can be incorporated into the compositions of the invention.

5           This invention further relates to a method of making nanoparticles containing a diagnostic or therapeutic agent having a block copolymer linked to at least one anionic group as a surface modifier adsorbed on the surface thereof, comprises of contacting the  
10   diagnostic or therapeutic agent with the sulfated nonionic block copolymer for a time and under conditions sufficient to form a stabilized nanoparticle. Contacting can be by admixing a  
15   suspension of the diagnostic or therapeutic agent with a solution of the block copolymer such as described above.

The following examples further illustrate the invention.

Example 1.       Preparation of Sulfated T908

20           T908 (25 g) was dissolved in 1,2-dichloroethane (500 ml) and chlorosulphonic acid (0.43 ml, 6 molar equivalents) was added. After 10 min of vigorous stirring, the solution was heated to 50°C and this temperature was maintained for 2.5 hours. On  
25   cooling the solvent was evaporated and the residue dissolved or dispersed in water (400 ml) and the pH adjusted to a value of approximately 10 by addition of dilute aqueous sodium hydroxide. The water was removed by heating on a steam bath (or freeze-drying). The  
30   residue was dissolved in methanol and the insoluble inorganic salts were filtered off. The methanol was removed by distillation under reduced pressure, and the T908 tetrasulphate dried in a vacuum oven (T, <40°C). Elemental microanalysis verified that the sulfur

-18-

content was consistent with 4 sulfate groups per molecule.

Example 2. Preparation of Sulfated F108

Pluronic F108 (35 g) was dissolved in 1,2  
5 dichloroethane (200 ml). Cholosulfonic acid (0.7 ml, 4  
molar equivalent) was added and the mixture heated at  
45-50°C for 3 hr. On cooling, the solvent was removed  
by evaporation at reduced pressure. The residue was  
dissolved in warm water (40-50°C, 200 ml or minimum  
10 possible) and adjusted to -pH 10 with sodium hydroxide  
solution. Ethanol (>1000 ml) was added and the  
solution evaporated under reduced pressure. Further  
additions of ethanol with evaporation gave a white  
solid which was dried at 40°C in the vacuum. Elemental  
15 microanalysis verified that the sulfur content was  
consistent with 2 sulfate groups per molecule.

Example 3. Preparation of Sulfated B20-3800

Polybutylene oxide, polyethylene oxide  
copolymer B20-3800 (8.8 g) was dissolved in 1,2  
20 dichloroethane (200 ml). The solution was purged with  
nitrogen gas for 20 mins. followed by the addition of  
chlorosulfonic acid (0.7 ml, 4 molar equivalent). The  
mixture was heated at 45-50°C for 3 hours under  
nitrogen atmosphere. On cooling, the solvent was  
25 removed by evaporation at reduced pressure. The  
residue was dissolved in warm water and adjusted to -pH  
10 with sodium hydroxide followed by work up procedures  
as used in example 2 to afford B20-3800 disulfate.  
Elemental microanalysis verified that the sulfur  
30 content was consistent with 2 sulfate groups per  
molecule.

-19-

Example 4. Preparation of Sulfated B20-5000

The method described in Example 4 was repeated except that polybutylene oxide, polyethylene oxide copolymer B20-5000 (11.6 g) was used in place of the copolymer B20-3800. Elemental analysis verified the presence of 2 sulfate groups per molecule.

Examples 5-12. Preparation of Nanocrystal Formulation and Autoclave Stability Evaluation

The following formulations were prepared at 15% diagnostic agent and 4% surfactant (w/v). A 6% stock solution was prepared by dissolving 600 mg of T908-tetrasulfate, F108-disulfate, B20-3800-disulfate or B20-5000-disulfate surfactants in 10 ml deionized water. To each 15 ml amber colored bottle, 7.5 ml ZrSiO<sub>4</sub> beads of size 1.1 mm, 562 mg of compound A (ethyl 3,5-diacetamido-2,4,6-triiodobenzoate) or compound B (6-ethoxy-6-oxohexyl-3,5-bis(acetamido)-2,4,6-triiodobenzoate), 2.5 ml of 6% stock surfactant solution and 0.994 ml deionized water were added. The sample bottle was sealed and placed on a roller mill running at 160 rpm for 5 days. At day 5, aliquot of samples were diluted 50 fold with deionized water for particle size measurement by Photon Correlation spectroscopy (Microtrac).

For terminal sterilization, 1 ml of nanocrystal formulations prepared above was pipetted into a 2 ml serum vial. After sealing with rubber septum and aluminum cap, the samples were subjected to autoclave at 121°C for 20 min. On cooling to room temperature, aliquot of samples were retreated from the vial and diluted 50 fold with deionized water for particle size measurement by Photon Correlation spectroscopy (Microtrac).

-20-

Example	Core	Surfactant	Mean Particle size (nm)	
			Before Auto.	After Auto.
5	Compound A	T908-tetrasulfate	154	233
6	Compound A	F108-disulfate	159	245
7	Compound A	B20-5000-disulfate	107	180
8	Compound A	B20-3800-disulfate	118	211
A	Compound A	F108	130	>500
B	Compound A	T8-908	241	>500
C	Compound A	B20-5000	106	>500
D	Compound A	B20-3800	110	>500
9	Compound B	T908-tetrasulfate	146	256
10	Compound B	F108-disulfate	145	233
11	Compound B	B20-5000-disulfate	110	199
12	Compound B	B20-3800-disulfate	110	213
E	Compound B	F108	130	>500
F	Compound B	T8-908	144	>500
G	Compound B	B20-5000	102	>500
H	Compound B	B20-3800	94	>500

Compared to the corresponding commercially available copolymeric surfactants under identical

-21-

milling and autoclave conditions, this data demonstrates that the sulfated block copolymers alone resulted in unexpectedly limited increases in particle size during terminal sterilization of nanocrystal formulations.

In addition, tail vein injection of a 4% solution of T908-disulfate, F108-disulfate and B20-5000-disulfate at 30 ml/Kg was well tolerated by mice.

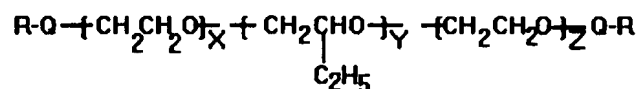
T908-tetrasulfate, F108-disulfate, B20-5000-disulfate also significantly inhibited the uptake of polystyrene particle by macrophages while B20-3800-disulfate had less effect. The degree of minimal macrophage uptake was found to be as follows: F108-disulfate > T908-tetrasulfate B20-5000-disulfate > B20-3800-disulfate.

The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

-22-

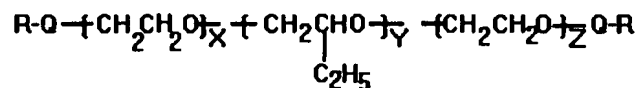
CLAIMS

1. A composition comprised of nanoparticles containing a therapeutic or diagnostic agent having a block copolymer linked to at least one anionic group as a surface modifier adsorbed on the surface thereof.
2. The composition of claim 1 wherein said anionic group is selected from the group consisting of sulfate, sulfonate, phosphonate, phosphate and carboxylate.
3. The composition of claim 1 wherein said polymer has a molecular weight of 1,000-50,000.
4. The compositions of claim 1 wherein said surfactant is a triblock copolymer having the structure



wherein Q is an anionic group, R is H or a metal ion, X and Y are 15-700; and Z is 5-200.

5. The composition of claim 1 wherein said surfactant is a triblock copolymer having the structure



wherein a is an anionic group, R is H or a metal ion, X and Y are 15-700; and Z is 5-200.

6. The composition of claim 1 wherein said polymer comprises at least 50% by weight of ethylene oxide units.
7. The composition of claim 1 wherein said diagnostic agent is ethyl 3,5-diacetoamido-2,4,6-triiodobenzoate.



-23-

8. The composition of claim 1 wherein said diagnostic agent is 6-ethoxy-6-oxohexyl-3,5-bis(acetamido)-2,4,6-triiodobenzoate.

9. A method of making nanoparticles containing a  
5 diagnostic or therapeutic agent having a block  
copolymer linked to at least one anionic group as a  
surface modifier adsorbed on the surface thereof  
comprised of contacting said diagnostic or therapeutic  
agent with said nonionic sulfated block copolymer for a  
10 time and under conditions sufficient to form a  
stabilized nanoparticle.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/15173

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K9/14 A61K9/51 A61K49/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 499 299 (STERLING WINTHROP INC.) 19 August 1992 see the whole document see page 9; example 7 see page 10; examples 11,12 ---	1-9
Y	US,A,4 539 012 (OHZEKI ET AL.) 3 September 1985 see column 6 - column 7; examples 6-14 ---	1-9
Y	EP,A,0 002 033 (BASF AKTIENGESELLSCHAFT) 30 May 1979 see the whole document ---	1-3,6-9
A	US,A,4 304 691 (FARMER, III ET AL. ) 8 December 1981 see the whole document -----	1-9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

12 April 1996

Date of mailing of the international search report

26. 04. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Benz, K

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/15173

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-499299	19-08-92	US-A- 5145684	08-09-92
		AU-B- 642066	07-10-93
		AU-B- 1014592	30-07-92
		AU-B- 654836	24-11-94
		AU-B- 1014792	30-07-92
		EP-A- 0498482	12-08-92
		IL-A- 100755	08-12-95
		JP-A- 4317053	09-11-92
		JP-A- 4295420	20-10-92
		US-A- 5451393	19-09-95
		US-A- 5494683	27-02-96
		US-A- 5399363	21-03-95
		US-A- 5318767	07-06-94
US-A-4539012	03-09-85	JP-B- 1030862	22-06-89
		JP-C- 1549662	09-03-90
		JP-A- 59174647	03-10-84
		CA-A- 1219540	24-03-87
EP-A-2033	30-05-79	DE-A- 2751519	23-05-79
		US-A- 4218218	19-08-80
		US-A- 4189445	19-02-80
US-A-4304691	08-12-81	CA-A- 1149287	05-07-83